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ORIGINAL PAPER

Comparative respiratory strategies of subterranean and fossorial octodontid rodents to cope with hypoxic and hypercapnic atmospheres

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Abstract Subterranean rodents construct large and complex burrows and spend most of their lives underground, while fossorial species construct simpler burrows and are more active above ground. An important constraint faced by subterranean mammals is the chronic hypoxia and hypercapnia of the burrow atmosphere. The traits, regarded as "adaptations of rodents to hypoxia and hypercapnia", have been evaluated in only a few subterranean species. In addition, well-studied subterranean taxa are very divergent to their sister groups, making it difficult to assess the adaptive path leading to subterranean life. The closely related sister genera *Octodon* and *Spalacopus* of Neotropical rodents offer a unique opportunity to trace the evolution of physiological mechanisms. We studied the ventilatory responses of selected octodontid rodents to selective pressures imposed by the subterranean niche under the working hypothesis that life underground, in hypoxic and hypercapnic conditions, promotes convergent physiological changes. To perform this study we used the following species: *Spalacopus cyanus* (the subterranean coruros) and *Octodon degus* (the fossorial degus) from central Chile. Ventilatory tidal volume and respiratory frequency were measured in non-anaesthetized spontaneously breathing animals. Acute hypoxic challenges (O_2 1–15%) and hypercapnia (CO_2 10%) were induced to study respiratory strategies using non-invasive whole body pletismography techniques. Our results show that coruros have a larger ventilatory response to acute hypoxia as than degus. On the other hand, hypercapnic respiratory responses in coruros seem to be attenuated when compared to those in degus. Our results suggest that coruros and degus have different respiratory strategies to survive in the hypoxic and hypercapnic atmospheres present in their burrows.

Keywords Ventilatory response · Hypoxia · Hypercapnia · Octodontids · Rodents

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Introduction

The convergent evolution of subterranean mammals is one of the most fascinating and puzzling evolutionary phenomena (Nevo 1999). Eight lineages of rodents have invaded the subterranean niche independently (Lacey et al. 2000); these species live most of their lives underground in their usually closed burrows, and they are called collectively subterranean. Other lineages have species that construct and use burrows, but are more active above ground, and they are called fossorial. Life underground imposes particular selective pressures, which have driven subterranean rodents to develop convergent morphological and physiological features (Nevo 1999). In comparison, fossorial rodents are much less specialized than subterranean species.

An important constraint faced by subterranean mammals is the low availability of O_2 (hypoxia) and the excess of CO_2 (hypercapnia) in the subterranean environment (Darden

1972; Arieli 1990; Shams et al. 2005). Maximal CO_2 levels (6.1%) and minimal O_2 levels (7.2%) were recorded in northern Israel in the breeding mounds of *Spalax carmeli* in a flooded, poorly drained field of heavy clay soil with high water content (Shams et al. 2005). Gas interchange between burrows and atmosphere depends on the gas permeability properties of the soil, which is affected by such factors as temperature and humidity (Arieli 1979), and the limited ventilation is the result primarily of animal movements (Buffenstein 2000). These factors make burrow gas composition to differ considerably from atmospheric air, and any activity of the inhabitants increases such difference. Models of diffusion gas exchange (Withers 1978) and experimental data (MacLean 1981) show that, unless the soil is completely devoid of biotic substances, burrow atmospheres will always be hypoxic and hypercapnic relative to the surface atmosphere (Buffenstein 2000).

Under low O_2 partial pressure (pO_2) in the burrow atmosphere and facing the potential CO_2 perturbation of their blood acid–base balance, subterranean mammals are expected to show certain physiological mechanisms to avoid excessive energy expenditure in respiratory work. These mechanisms are not fully understood, but include several physiological adjustments in relation to the predicted values of mammals of similar body mass (predicted by Stahl 1967) and at different levels (reviewed by Boggs et al. 1984; Buffenstein 2000; Nevo 1999). For example, the critical pO_2 levels in these mammals are lower (Arieli 1990; Arieli and Nevo 1991; Lechner 1977), and metabolic rates can be maintained at low pO_2 (Lovegrove 1989; Vleck 1979). Besides, ventilation in normoxia is lower (Arieli 1990; Boggs et al. 1998), and ventilatory response to hypoxia, hypercapnia, or both is attenuated (Arieli 1990; Boggs et al. 1998; Buffenstein 2000). Cardiac output and heart frequency are also lower in normoxia (Arieli 1990), and show a twofold increased response to hypoxia even at low temperatures (Arieli et al. 1986). Moreover, oxygen carrying capacity is increased, facilitated by elevated hemoglobin concentrations, high intrinsic affinity for oxygen and more red blood cells (reviewed by Buffenstein 2000). Furthermore, their myoglobin concentration is higher in blood and skeletal muscles; the capillary density in myocardium, skeletal muscles, and lungs is elevated (Arieli 1990), and they have a high relative amount of mitochondria in skeletal muscles (Widmer et al. 1997). Finally, they have high arterial and tissue pCO_2 (Arieli 1990), and they cope with it without altering the blood acid–base equilibrium by at least two known different mechanisms (Buffenstein 2000; Quilliam et al. 1971).

Other mechanisms are known only for species of the genus *Spalax* and are related to shortening of the diffusion distance, increase of the O_2 permeability constant (reviewed by Arieli 1990), and avoidance of oxidative damage under fluctuating O_2 and CO_2 levels by increasing the activity levels of antioxidant enzymes, such as superoxide dismutase (Caballero et al. 2006). Recent studies have also found differences in gene expression among subterranean species with different critical pO_2 (Avivi et al. 2006; Nasser et al. 2005; Polyakov et al. 2004). However, these mechanisms have been evaluated in only a few subterranean species from the genera *Spalax* (Mediterranean), *Heterocephalus* (African), and *Thomomys* (North American), and there are no comparative studies including several species. Besides, well-studied subterranean taxa are very divergent from their sister groups (if known), making it difficult to assess the adaptive path leading to subterranean life.

To understand the physiological strategies of subterranean mammals for coping with subterranean atmospheric conditions in comparison to less specialized forms, we examined the influence of experimental variations in hypoxic and hypercapnic conditions on respiratory tidal volume and respiratory frequency in response to different values of pO_2 and pCO_2, of the fully subterranean coruro *Spalacopus cyanus* (Rodentia: Octodontidae) and the fossorial degu *Octodon degus* (Rodentia: Octodontidae). Coruros are distributed from 30 to 37°S along the coast of Chile and also above 2,000 m sea level in the Andes range. Degus are diurnal rodents inhabiting the semiarid and Mediterranean environments of northern and central Chile to 2,000 m above sea level. In many areas of their range of distribution, both species overlap and are sympatric, but one inhabits above (degus) and the other below (coruros) the surface. We hypothesized that coruros show an attenuated response to hypercapnia and hyperoxia compared with the related degus, whereas the response to hypoxia is depressed in the latter.

The closely related sister genera *Octodon* and *Spalacopus* of Neotropical endemic rodents offer an opportunity to trace the evolution of physiological traits. The monotypic genus *Spalacopus* is strictly subterranean and shares convergent adaptations to underground life, while the genus *Octodon* has three burrowing species. Moreover, the phylogenetic relationships and estimations of divergence times are well established as relatively recent (reviewed by Opazo 2005), which would allow tracing the changes associated with the acquisition of subterranean adaptations along a known phylogeny, as well as identifying and discriminating such adaptations from more general ones associated with fossoriality.

Methods

Animals studied

Experiments were performed on five adults of the fossorial species *Octodon degus* ("degus"; all males between 194.4

and 217.8 g) and four adults of the subterranean species *Spalacopus cyanus* ("coruros"; 3 males and 1 female between 72.8 and 100.4 g). Degus and coruros were caught using Oneida Victor Traps and Tomahawk Live Trap traps, respectively, at a field station of the Universidad de Chile (33°23′S, 70°31′W, altitude 495 m) in Rinconada de Maipú, in the Metropolitan Region, Chile, between October 18th and October 21st, 2007. Traps did not injure the animals, all of which were non-reproductive and looked completely healthy. Animals were kept in a ventilated room in individual polycarbonate rat cages (45 × 23 × 21 cm) with a bedding of hardwood chips, water, and food (rabbit commercial pellet supplemented with apples, carrots, lettuce, and sunflower seeds) provided ad libitum, with a photoperiod of LD = 12:12 and ambient temperature set at 25°C. Animals were kept under these conditions approximately 10 days before measurements began. After measurements were made, animals were entered into collection of Pontificia Universidad Católica de Chile with the access numbers SSUC_Ma 00408–004012 and SSUC_Ma 00412–00415 for coruros and degus, respectively.

Measurements

The experimental protocol was approved by the Ethical Committee of the Facultad de Ciencias Biológicas of the Pontificia Universidad Católica de Chile. We measured tidal volume (V_T, ml), respiratory frequency (f_R, breaths per minute, BPM), minute inspiratory volume (V_I, ml/min) in response to different pO_2 (from anoxia to hyperoxia) and one pCO_2 level using a whole body pletismography system (Respiromax, Columbus Instruments, USA) in conscious normally breathing animals. The system includes a restrainer with a design that allows an unobtrusive, but secure seal around the animal's neck that isolates the head in the inhalation/exposure chamber. The calibration of the experimental setup was performed before every measure using the value of the barometric pressure at the level of Santiago city at each day of experiment. We measured the ventilatory responses elicited by poikilocapnic levels of pO_2 (5–670 Torr), maintained until the response was in a semi steady state (~10–20 s).

The experimental conditions sequentially tried were: (1) normoxia and normocapnia (room air), (2) anoxia (100% N_2 for 20 s), (3) 30 hypoxia (pO_2 5–100 Torr for 30 s), (4) hyperoxia (100% O_2 for 60 s), and (5) hypercapnia (air with 10% CO_2 for 30 s). Enough time was allowed between measurements until basal parameters were reached in normoxia. Animals were exposed to 100% O_2, which is the well known Dejours (1962) test. Because of its non-invasive nature, the Dejours test is used in human subjects and animals to assess the peripheral chemoreceptors contribution to ventilation. Exposure to hyperoxic (100% O_2) stimuli leads to ventilatory depression that occurs during the first few seconds of hyperoxia (15–20 s). This effect is primarily due to a decrease in the carotid body chemosensory activity. The magnitude of the ventilatory depression caused by hyperoxia is often used as an index of carotid body chemoreception sensitivity.

The hypoxic mixtures were obtained by mixing 100% O_2 and 100 N_2 using a flowmeter system (Dwyer Instruments, USA). Optimal values for hypoxic mixtures were measured using an oxygen analyzer (Omehda, USA). In all cases, experiments were performed at room temperature, between 25 and 27°C.

Statistical analysis

Respiratory frequency, tidal volume, and minute inspiratory volume are expressed as mean ± SEM unless otherwise stated. For comparisons between species, the respiratory data were normalized with respect to basal values. A two-way analysis of variance (ANOVA) was performed to seek for statistical difference between species and different pO_2 treatments. An unpaired t test with Welch's correction was performed to seek for statistical difference of hypercapnia and hyperoxia between species.

Results

Mean basal values of f_R, V_T, and V_I, and their response to different inspired pO_2 and hypercapnia are summarized in Table 1. Figure 1 shows the variation of the three variables to different pO_2 tried. Figure 2 shows these variables in response to 10% of CO_2.

Ventilation in normoxia

According with expectations regarding their larger body mass, degus have higher V_T (0.18 ± 0.04 ml) and V_I (40.60 ± 11.65 ml/min) than coruros ($V_T = 0.12 \pm 0.03$ ml and $V_I = 18.70 \pm 4.57$ ml/min). Interestingly, degus have also higher f_R than coruros (230.68 ± 34.52 and 157.65 ± 14.38 BPM, respectively). However, neither of these differences is statistically significant ($P > 0.10$ in all cases). Both species have significantly higher f_R and lower V_I and V_T than expected for their body mass according to Stahl (1967), compared to expected values f_R: 287 and 153%, V_T: 12 and 21%, V_I: 38 and 28%, in degus and coruros, respectively.

Effect of hypoxia

Both species respond to hypoxia by increasing f_R, V_T, and V_I. However, in degus, the ventilatory response to different

Table 1 Variation of respiratory variables in response to different pO_2 and pCO_2

	Normoxia	Anoxia	Hypoxia			Hyperoxia	Hypercapnia
	21% O_2	100%N_2	5% O_2	10% O_2	15% O_2	100% O_2	10% CO_2
Respiratory frequency							
Degus	230.68 ± 34.52	235.42 ± 6.64	201.22 ± 19.33	179.31 ± 17.05	166.11 ± 16.12	135.41 ± 20.10	205.5 ± 20.4
		[1.02 ± 0.01]	[1.06 ± 0.01]	[0.94 ± 0.02]	[0.93 ± 0.04]	[0.82 ± 0.10]	[1.24 ± 0.10]
Coruros	157.65 ± 14.38	173.78 ± 1.11	171.14 ± 1.11	158.91 ± 1.11	152.17 ± 1.07	90.43 ± 0.61	155.95 ± 1.03
		[8.50 ± 0.05]	[19.29 ± 0.04]	[11.27 ± 0.04]	[8.36 ± 0.03]	[17.21 ± 0.11]	[9.11 ± 0.05]
Tidal volume							
Degus	0.18 ± 0.04	0.21 ± 0.04	0.22 ± 0.04	0.17 ± 0.04	0.15 ± 0.04	0.09 ± 0.02	0.16 ± 0.03
		[1.26 ± 0.12]	[1.34 ± 0.10]	[1.31 ± 0.15]	[1.12 ± 0.05]	[0.73 ± 0.03]	[1.32 ± 0.16]
Coruros	0.12 ± 0.03	0.18 ± 0.04	0.17 ± 0.02	0.14 ± 0.03	0.13 ± 0.02	0.09 ± 0.02	0.15 ± 0.03
		[1.54 ± 0.10]	[1.51 ± 0.20]	[1.45 ± 0.17]	[1.12 ± 0.05]	[0.87 ± 0.11]	[1.21 ± 0.08]
Minute volume							
Degus	40.60 ± 11.65	49.46 ± 12.29	45.44 ± 12.14	32.31 ± 10.24	23.87 ± 8.28	13.83 ± 5.02	34.56 ± 7.97
		[1.28 ± 0.12]	[1.42 ± 0.12]	[1.23 ± 0.14]	[1.03 ± 0.05]	[0.60 ± 0.07]	[1.84 ± 0.10]
Coruros	18.70 ± 4.57	29.50 ± 4.34	28.40 ± 4.75	22.28 ± 4.01	18.90 ± 2.72	7.90 ± 2.01	22.65 ± 4.70
		[1.71 ± 0.19]	[1.64 ± 0.15]	[1.63 ± 0.26]	[1.20 ± 0.08]	[0.50 ± 0.03]	[1.26 ± 0.05]

In each cell, mean values of respiratory variables (respiratory frequency in BPM, tidal volume in ml, and minute volume in ml/min) ± SEM to different pO_2 and pCO_2, above, and below between brackets its mean response with respect to basal values ± SEM. Degus and coruros are *O. degus* and *S. cyanus*, respectively

pO_2 was lower (Fig. 1). We found a statistically significant difference between the V_I response (accounts for approximately 4.84% of the total variance, $F = 7.5_{(1,42)}$, $P = 0.009$) and treatments of different pO_2 (approximately 65.83% of the total variance, $F = 20.42_{(5,42)}$, $P < 0.0001$). Increments in V_T seem to be more evident than those in f_R, and the latter seem to be more important in coruros than in degus. In the case of V_T, pO_2 accounts for approximately 52.48% of the total variance and is extremely significant ($F = 10.84_{(5,41)}$, $P < 0.0001$) and species effect accounts for approximately 3.88% of the total variance and is marginal ($F = 4.01_{(1,41)}$, $P = 0.0519$). In the case of f_R, species effect accounts for approximately 1.54% of the total variance, but is not significant ($F = 1.98_{(1,41)}$, $P = 0.1673$), whereas pO_2 accounts for approximately 57.93% of the variance and is extremely significant ($F = 14.82_{(5,41)}$, $P < 0.0001$). Statistically significant interaction between species and pO_2 was only found for f_R (approximately 15.19% of the total variance $F = 3.89_{(5,41)}$, $P = 0.0056$), so the P values of species and pO_2 effects are more difficult to interpret in this case.

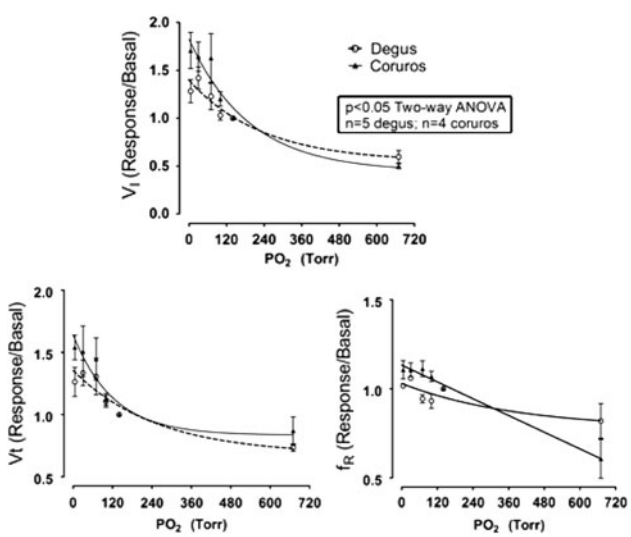

Fig. 1 Variation of the respiratory variables to different pO_2. All variables are represented as its mean response with respect to basal values ± SEM; f_R respiratory frequency in BPM; V_T tidal volume in ml; V_I in ml and minute volume. Degus and cururos are *O. degus* and *S. cyanus*, respectively

Effect of hypercapnia

Both species respond to hypercapnia by increasing both V_T and f_R. The response was large in degus than in coruros (Fig. 2). There was no difference between species in the response of V_T and f_R (with respect to basal values) to 10% of CO_2, whereas the response of V_I (with respect to basal values) was marginal ($t = 2$, $df = 5$, $P = 0.0847$).

Effect of hyperoxia

In response to 100% O_2, both species showed a decrease both in V_T and f_R (and consequently V_I), but the response was more accentuated in coruros than in degus, and

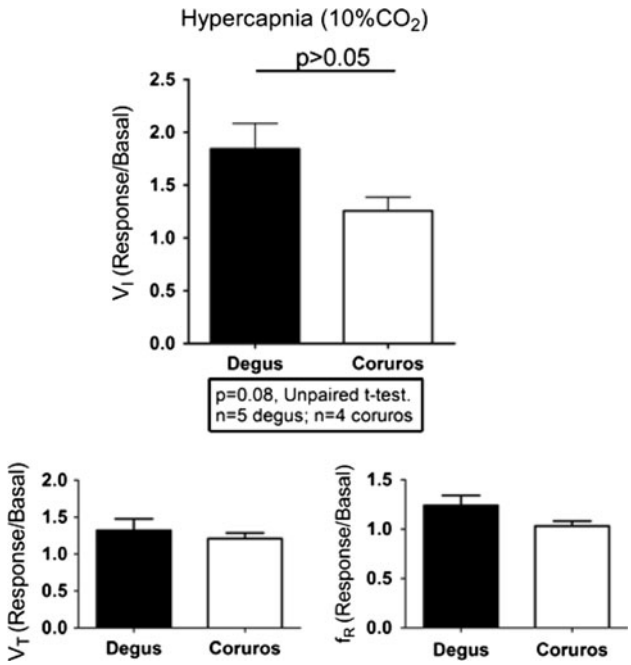

Fig. 2 Respiratory variables in response to 10% of CO_2. All variables are represented as its mean response with respect to basal values ± SEM; f_R respiratory frequency in BPM; V_T tidal volume in ml; V_I in ml and minute volume. Degus and cururos are *O. degus* and *S. cyanus*, respectively

specially by a reduction of f_R. However, neither of these differences between species is statistically significant ($P > 0.10$ in all cases).

Discussion

Mammalian respiration increases in response to hypoxia and/or hypercapnia. However, the ventilatory responses of high altitude, fossorial, or diving mammals may differ from this scheme (Buffenstein 2000; Ramírez et al. 2007; Nevo 1999). It is generally accepted that burrowing mammals show a reduced ventilatory sensitivity in particular to hypercapnia and possibly to hypoxia (Boggs et al. 1984).

This is the first time that a study addresses the ventilation response to hypoxia and hypercapnia in closely related octodontid rodents, including one fossorial species and one strictly subterranean species. Coruro (*S. cyanus*) was chosen because it is exposed to hypoxic and hypercapnic ambient in its complex burrows. Degus (*O. degus*) is a closely related species that maintains semifossorial habits and uses burrows, but it is cursorial with daily activity outside their burrows (Vásquez et al. 2002) and frequent access to open air. Our results show that both species have different ventilation responses to hypoxia and hypercapnia, suggesting different physiological adaptations to the hypoxic and hypercapnic environment.

As expected, given their larger body mass, degus have had higher observed values of V_T and V_I in normoxia than coruros, although they are not significant, possibly due to the small sample sizes used. Interestingly, both species had significantly higher f_R, and lower V_I and V_T in normoxia than the expected for their body mass (following allometric scaling proposed by Stahl 1967). This result agrees with the idea that the reduced ventilation in normoxia is a characteristic physiological response among fossorial mammalian species to cope with hypoxic and hypercapnic atmospheres. It is interesting to note that f_R is higher (1.5 and 3 times) than that predicted by allometry for coruros and degus, respectively. Subterranean species *T. bottae* and *S. ehrenbergi* display normoxic ventilation 80 and 70%, respectively, compared to the one predicted (Arieli and Ar 1979; Darden 1972). As suggested by Arieli and Ar (1979), if normal mammalian gas transport is maintained at a low ventilation rate, the potential remains to increase ventilation and, together with other physiological traits, to maintain normal gas transport in the hypoxic-hypercapnic atmosphere of the burrow. Low oxygen consumption, together with the low ventilation in normoxia found in these species, demands other mechanisms along the gas transport pathway to maintain a standard metabolic rate, as has been shown for other species at several levels (e.g., cardiovascular, hematological, and tissue levels). Only few complementary strategies have been reported for these species, and some others should be investigated. It has been reported that coruros have a basal metabolic rate (75–85% lower than expected) and critical pO_2 levels significantly lower than the aboveground counterparts (Contreras 1986). Although comparisons between coruros and degus have not been done, data suggest that the critical pO_2 in coruros (ranged from 79.4 to 91.9 Torr) is lower than in degus (139 Torr) (Morrison and Rosenmann 1975; Rosenmann and Morrison 1975; Contreras and McNab 1990). Data on resting metabolic rate reported in these studies are not easily comparable. On the other hand, hematocrit and the erythrocyte hemoglobin concentration are reported only for degus (48.8 ± 6% and 256 ± 4 g/L, respectively), but they did not show a particular pattern (Morrison et al. 1963). The low normoxic ventilation of coruros and degus may be an example of convergent evolution to the fossorial niche in rodents, which is more accentuated in subterranean life.

Contrasting the results from the two closely related species analyzed in this study, give clues that uncover not only the action of selective pressures of subterranean habitat but also difference in chemoreceptors sensibility. Coruros showed an attenuated response to hypercapnia and hyperoxia compared with degus, meanwhile the response to different levels of hypoxia seems to be depressed in the latter. This result strongly suggests that subterranean

rodents have a blunted ventilatory response to hypercapnia. It is generally accepted that burrowing mammals show a reduced ventilatory sensitivity in particular to hypercapnia and possibly to hypoxia (Boggs 1992; Boggs et al. 1984; Tenney and Boggs 1986). The "actual" respiratory pattern may be masked in these marginal significances due to the low numbers of individuals available for this study. Unfortunately, no complementary data on these species are available yet to support our findings. So, although these results must be viewed with caution, they suggest that adaptative physiological changes in response to extreme hypoxia, characteristic of the subterranean niche, have appeared recently in the lineage leading to *Spalacopus*, no longer than 2.5 millon years ago (Lessa et al. 2008). Previously studied subterranean rodents have deep roots that prevent us from determining the time elapsed from these acquisitions.

Reduced ventilatory response to hypoxia presented by degus in comparison to coruros is not the pattern expected initially, but it is not necessarily a characteristic of subterranean mammals, and is found in some burrowing animals. In studies on the echidna *Tachyglossus aculeatus* (Frappell et al. 1994), the armadillo *Dasypus novemcincus* (Boggs et al. 1998), and the wombat *Lasiorhinus latifrons* (Frappell et al. 2002), the hyperventilatory response is depressed under hypoxia. However, other species like the Syrian hamster *Mesocricetus auratus* (Walker et al. 1985), woodchuck *Marmota monax* (Boggs and Birchard 1989), and golden-mantled squirrel *Spermophilus lateralis* (Barros et al. 2001) and columbian ground squirrel *S. columbianus* (Milsom et al. 1986), which are all fossorial, show no difference from similar-sized epigeal species. In fossorial golden-mantled ground squirrel *Spermophilus lateralis*, a biphasic response to hypoxia of f_R has been reported, with an initial increase followed by a decline back to resting levels (Barros et al. 2001).

The fact that subterranean coruros have a more acute response to hypoxia than fossorial degus, which is in agreement with previous studies which show how persistent changes in the neural control system are generated based on prior experience (Mortola 2004). Chronic sustained hypoxia (pO_2 50–70 Torr) elicits plasticity in the carotid body chemoreceptors (Mitchell and Johnson 2003), with delayed effects on the central neural integration of carotid chemoafferent neurons that become more prominent as the duration of hypoxia is extended (Dwinell and Powell 1999; Powell et al. 1998). In the case of coruros, hypoxia may last long periods inside closed burrows, and the species show enhanced ventilatory response as the result of the potentiation of the carotid chemoreflex to this stimulus. However, hypoxia during the neonatal period affects adult ventilatory control, altering resting breathing patterns, and attenuating the hypoxic ventilatory response (Okubo and Mortola 1990). On the other hand, degus might tolerate a likely intermittent hypoxia throughout their lives, facing hypoxia only while resting at night. This stimulation may elicit plasticity via central neural mechanisms (Ling et al. 2001) with additional effects at the carotid body chemoreceptors (Prabhakar 2001; Rey et al. 2004), which may increase the short-term hypoxic ventilatory response (Ling et al. 2001) observed in the species.

The ventilatory response to changes in inspired CO_2 is an increase in ventilation, either in the rate or the depth of inhalation. This response as well as CO_2 sensitivity displays considerable interspecific variation (Boggs et al. 1998), but appears to be linked to habitat. Burrowing animals often encounter hypercapnia, and have on average a reduced sensitivity to CO_2 and reduced response to it compared to their non-fossorial or non-diving counterparts (reviewed by Boggs et al. 1984; Frappell et al. 2002), despite some exceptions (*Proechimys yonenagae:* Barros et al. 1998; *Spermophilus lateralis:* Garland et al. 1994). Increased tidal volume in response to CO_2 is a consistent response interspecifically within eutherian mammals (Barros et al. 1998; Frappell et al. 2002), with a few notable exceptions related to anatomical constraints which increase only the f_R (the armadillo *Dasypus novemcincus* (Boggs et al. 1998) and the bat *Phyllostomus discolor* (Walsh et al. 1996)) as explained by Frappell et al. 2002, or the subterranean *S. ehrenbergi* which simultaneously increase V_T and f_R (Arieli and Ar 1979). Wang and Warburton (1995) proposed that, in animals that employ the diaphragm muscle to shift the liver posteriorly and thereby expand the lungs, increasing ventilation by increasing tidal volume is less expensive than by increasing frequency. The response of coruros to CO_2 is similar to the general one reported for burrowing mammals: an attenuated increase in ventilation mediated primarily through changes in tidal volume. This result may support convergent evolution among subterranean rodents that have invaded the underground life independently. On the other hand, degus strongly respond to CO_2 by increasing both respiratory variables, and this pattern differs from predictions for fossorial mammals.

Response to hyperoxia was more attenuated in degus than in coruros. O_2-sensitive chemoreceptors are external to the brain in the carotid bodies (instead CO_2/pH-sensitive chemoreceptors are in the carotid bodies, but major sites are also distributed within the brain). The hyperoxic Dejours (1962) test has been extensively used to establish peripheral carotid body chemoreceptor sensitivity to oxygen. Coruros showed a dramatic decrease in f_R in response to 100% O_2 compared to degu's ventilatory response. These results suggest that cururos show an increase in response to acute hypoxia, due to an enhanced oxygen peripheral chemoreceptor sensibility. Unfortunately, the response to hyperoxia has not been studied in other

subterranean/fossorial taxa, and it is not possible to compare our results with other species.

Several limitations of these studies come from the way of working in this field. Comparisons with other species studied are difficult, because previous studies have been carried out under very different conditions of pO_2 and pCO_2, either in time or in concentration of each gas. It would be informative to standardize the results from different species to be able to compare and obtain general patterns. In this study, a non-invasive method was used, in which animals were not anesthetized during the experiments. This has the advantage of not altering stimulation and natural chemoreception, but it includes the effect of stress of animals and the possible acclimatization of the animal to the systems. These results cannot be directly compared with most of the previously reported data because they used anesthetized animals, so these kind of complementary studies in degus and coruros are needed. Another drawback of these studies is the lack of comparisons within a proper phylogenetic context. Most of the conclusions taken in previous studies came from comparing a fossorial species with a distant phylogenetically related laboratory rat. Finally, the separate effects of hypoxia and hypercapnia are important to characterize the response to a single stimulus. However, this approach cannot predict the effect of two stimuli together. In future studies, different levels of hypoxia and hypercapnia together may be used in order to assess the combined effect of both stimuli.

In conclusion, both degus and coruros have lower ventilation in normoxia than expected for their body mass, according to fossorial/burrowing habits, but they differ in their response to hypoxia, hyperoxia, and hypercapnia. Coruros, but not degus, exposed to hypercapnia showed a blunted hyperventilatory response, characteristic of fossorial mammals. Exposure to hypoxia resulted in greater hyperventilation in cururos than in degus, suggesting that cururos show a greater sensitivity to changes in oxygen concentration via peripheral chemoreceptors.

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